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ON THE MECHANISM OF OPSONIC ACTION.*

LUDVIG HEKTOEN.

(From the Memorial Institute for Infectious Diseases, Chicago.)

ACTIVATION OF HEATED OPSONIC SERUM.

NUMEROUS investigators have found that the total opsonic action of fresh serum is the result of the combined action of two distinct bodies, one thermostable and the other thermolabile. In other words, opsonins have the same general constitution as lysins. There is also practically general agreement to the effect that the thermostable body, i.e., the body which persists after serum has been heated to 55° C. or so for about 30 minutes and is absorbable at 0° C., by itself is capable of opsonic action. Heated normal serum, however, contains comparatively little of thermostable opsonic substance, whereas immune serum may be very rich in this body. Driven by the con-conception that opsonins are different from the tropins (as the substances that promote phagocytosis in immune serum have been called) Neufeld[1] would deny that heated normal serum has any power to promote phagocytosis except in special instances. This is not in accord with the observations with which I am familiar as mixtures containing heated normal serum (58–60° C. for 30 min.) have always given greater phagocytosis than mixtures containing salt solution only. It is true that accurate numerical methods sometimes may be necessary to detect the difference, but the difference is there and unmistakable. It should be stated that according to Hamilton's[2] investigations the opsonins for certain pseudodiphtheria bacilli in the serum of rabbits and goats, normal as well as immune, are simple thermostable substances, those in immune serum consequently corresponding closely to Neufeld's bacteriotropins. In Eggers'[3] hands the serum from some persons proved to contain inactivable thermostable opsonic elements at least with respect to the pneumococcus and streptococcus. This observation is of interest as it may explain why the earlier

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[1] Kolle and Wassermann's *Handbuch*, 1908, Ergänzungsband 2, p. 313.

[2] *Jour. Infect. Dis.*, 1908, 5, p. 570.

[3] *Ibid.*, 1908, 5, p. 263.

attempts by Hektoen and Ruediger[1] and Bullock and Atkin[2] at re activation proved failures.

Most of the experiments the results of which have led to the conclusion that two substances co-operate in producing the opsonic effect of many sera have been made with bacteria. Neufeld and Bickel[3] have demonstrated well the interaction of thermostable and thermolabile substances in the production of phagocytosis of red corpuscles, but they interpret this opsonic effect as caused by the lytic amboceptor and complement. Consequently further results obtained with red corpuscles as the object of opsonification may be of interest. In my experiments the mixtures contained equal quantities of 5 per cent suspension of carefully washed red corpuscles, of suspension of carefully washed leucocytes obtained from pleural exudate in a dog, produced by aleuronat, and of serum + salt solution, the total quantity being always the same. After one hour at 37° C., during which the tubes containing the mixtures were gently agitated by means of a shaking machine, smears were made and stained and the percentage of phagocytic leucocytes determined by counting a large number of leucocytes (100–400). Usually a larger percentage of mononuclears are phagocytic than of the polymorphonuclears, but unless otherwise stated the figures represent the gross percentage of phagocytic cells among all forms of leucocytes. The method, while by no means perfect, readily detects accurately enough material differences in the amount of phagocytosis in comparable mixtures. A brief statement of the result shown in Table 1 may be desirable: In doses of 0.001 c.c. the heated serum of a rabbit immunized to goat blood corpuscles proved only feebly opsonic for washed goat corpuscles, using dog leucocytes as phagocytes, but the addition of 0.01 c.c. of normal guinea-pig serum, of itself without demonstrable opsonic action, materially increased the amount of phagocytosis.

TABLE 1.

PHAGOCYTOSIS OF GOAT CORPUSCLES UNDER THE INFLUENCE OF IMMUNE RABBIT SERUM AND NORMAL GUINEA-PIG SERUM.

Immune Serum	Normal Guinea-Pig Serum	Phagocytosis
0.001		4
0.001	0.01	20
	0.01	0

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[1] *Ibid.*, 1905, 2, p. 129.

[2] *Proc. Roy. Soc.*, 1905, 74, p. 380.

[3] *Arb. kais. Gesundh.*, 1907, 27, p. 310.

Other illustrations of the same phenomenon are given in Tables 2, 3, 4, and 5. The tables show that the activating or stimulating element may be contained in the serum of some other animal than that furnishing the thermostable body. Table 5 gives an instance of this phenomenon on part of a normal serum (guinea-pig). Elsewhere[1] are given similar results obtained with heated and fresh dog serum and rat corpuscles.

TABLE 2.

PHAGOCYTOSIS OF HUMAN CORPUSCLES UNDER THE INFLUENCE OF IMMUNE (ANTIHUMAN) RABBIT SERUM AND NORMAL DOG SERUM.

Immune Serum	Normal Dog Serum	Phagocytosis
0.0013	....	16
0.0004	....	7
0.0013	0.02	50
0.0004	0.02	25
......	0.02	4

TABLE 3.

PHAGOCYTOSIS OF HUMAN CORPUSCLES UNDER THE INFLUENCE OF IMMUNE (ANTIHUMAN) RABBIT SERUM AND NORMAL HUMAN SERUM.

Immune Serum	Normal Human Serum	Phagocytosis
0.05	.....	2
0.05	0.012	9
0.05	0.006	10
....	0.012	1
....	0.006	0

TABLE 4.

PHAGOCYTOSIS OF RABBIT CORPUSCLES UNDER THE INFLUENCE OF IMMUNE AND NORMAL DOG SERUM.

Immune Serum	Normal Serum	Phagocytosis
0.012	....	15
0.004	....	8
0.012	0.02	35
0.004	0.02	10
.....	0.02	3

TABLE 5.

PHAGOCYTOSIS OF GOAT CORPUSCLES UNDER THE INFLUENCE OF HEATED AND UNHEATED GUINEA-PIG SERUM.

Heated Serum (60° 30 min.)	Fresh Serum	Phagocytosis
0.05	.....	0
0.025	.....	0
0.05	0.012	17
0.025	0.012	8
.....	0.012	0

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[1] Hektoen, *Jour. Infect. Dis.*, 1908, 5, p. 249.

The results of these experiments indicate that many hemopsonic sera, normal as well as immune, owe their full action to a thermostable opsonic substance and a thermolabile complement-like body which greatly promotes the action of the first substance. The results also suggest the query, why, if the opsonic action of normal serum really is produced by lytic amboceptor and complement as claimed by Neufeld, should we not consider the opsonic action of immune serum as owing to the lytic amboceptor and complement? So far as results of activation experiments go, normal and immune sera act alike, and the logical conclusion would seem to be that in both cases the opsonic action is the effect of the same bodies, be they identical or not with the lytic amboceptor and complement, and not of dissimilar bodies, as Neufeld would have it when he urges that the hemotropic power of normal serum be ascribed to the lytic amboceptor and complement and that of immune serum at least to a very large extent to a different kind of substance without any normal homologue, namely hemotropin. Neufeld assumes that the so-called cytotropins cannot be activated, and when he finds that small quantities of heated immune serum in itself without opsonic effect is activated by normal serum, he seems to make the further assumption that in this case too it is the lytic amboceptor which is activated. The theory meets difficulty too when we attempt to apply it to opsonic substances for bacteria like pneumococci and streptococci that are not subject to lysis by serum but readily opsonified. Neither does Neufeld's contention harmonize well with the fact that on the entrance of antigen into the body there occurs, as I have shown, a depression of the opsonic index which is specific for the antigen in question.[1] At present it seems to me the other view, namely that the specific opsonic substances in normal and immune serum are identical, differing only in quantity, harmonizes better with the established facts in regard to these substances as well as in regard to antibodies in general. Hamilton's simple thermostable opsonic substances correspond well to Neufeld's tropins but they occur in normal as well as immune serum.[2]

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[1] *Ibid.*

[2] Wright and Reid (Wright and Reid, *Proc. Roy. Soc.*, 1906, 77, p. 211) make objection to the term bacteriotropin as used by Neufeld, in the first place because better applicable to the whole class of substances which combine with bacteria and secondly because used by Wright in another sense from Neufeld's as early as 1899.

MECHANISM OF OPSONIC ACTIVATION.

The question arises, how does activation of heated opsonic serum take place? Is it possible to show by appropriate absorption experiments whether or not the thermolabile body is anchored to the corpuscle (or, in case of bacteriopsonin, to the bacterium) by means of the thermostable body, or perhaps in some other way? To this end the following experiments were devised. The consideration upon which they are based is this—that if the opsonic complement is fixed firmly to the corpuscle or bacterium its activating influence should persist after the corpuscle or bacterium treated with the sera in question is washed in salt solution. The first two experiments constitute an analysis in this way of the action of small quantities of normal serum;

TABLE 6.

ANALYSIS OF ACTION OF FRESH DOG SERUM IN PROMOTING PHAGOCYTOSIS OF RAT CORPUSCLES UNDER THE INFLUENCE OF HEATED DOG SERUM.

	Corpuscles (5% susp.)	Heated Dog Serum (59° 30 min.)	Fresh Serum (same as the heated)	NaCl Sol.
Tube 1	0.5 c.c.	0.1 c.c.	....	0.1 c.c.
Tube 2	0.5	0.1	0.03	0.07
Tube 3	0.5	1.0	....	0.1
Tube 4	0.5	...	....	0.2

After inoculation at 37° C. for 60 min. the corpuscles were washed twice in large quantities of NaCl solution and in each case there were added suspension of washed leucocytes 0.5 c.c. and NaCl sol. 0.1 c.c. **except that Tube 3 received fresh normal serum 0.03 in NaCl sol. 0.07 in place of NaCl sol. only.** After incubation under gentle shaking for 60 min. smears were made and the percentage of mononuclears determined with the following result:

Tube 1	1
Tube 2	1
Tube 3	**50**
Tube 4	0

TABLE 7.

ANALYSIS OF THE ACTION OF NORMAL GOAT SERUM IN PROMOTING PHAGOCYTOSIS OF SHEEP CORPUSCLES UNDER THE INFLUENCE OF IMMUNE GOAT SERUM.

	Corpuscles (5% Susp.)	Immune Serum (60° 30 min.)	Fresh Serum	NaCl Sol.
Tube 1	0.2 c.c.	0.0025 c.c.	....	In all sufficient to make the total quantity 0.4 c.c.
" 2	"	"	0.01 c.c.	
" 3	"	"	0.005	
" 4	"	"	....	
" 5	"	"	....	
" 6	"	......	....	
" 7	"	......	....	

After incubation at 37°C. for 60 min. the corpuscles were washed carefully, the supernatant fluid removed wholly, and the experiment completed by adding to each tube as follows, and incubating for one hour:

Tube 1. NaCl sol. 0.1+suspension of dog leucocytes 0.2	24*
“ 2. “ “ 0.1+ “ “ “ “ 0.2	22
“ 3. “ “ 0.1+ “ “ “ “ 0.2	20
“ 4. Nor. ser. 0.01 in NaCl sol. 0.1+susp. dog. leuc. 0.2	50
“ 5. “ “ 0.005 “ “ “ 0.1+ “ “ “ 0.2	44
“ 6. “ “ 0.01 “ “ “ 0.1+ “ “ “ 0.2	0
“ 7. “ “ 0.005 “ “ “ 0.1+ “ “ “ 0.2	0

* The figures give the percentage of mononuclears engaged in phagocytosis.

TABLE 8.

ANALYSIS OF ACTION OF NORMAL HUMAN SERUM IN PROMOTION OF PHAGOCYTOSIS OF COLON BACILLI UNDER INFLUENCE OF HEATED IMMUNE HUMAN SERUM.

	Bacillary Susp.	Immune Serum	Normal Serum	NaCl Sol.
Tube 1	0.3 c.c.	0.1 c.c.	....	In all sufficient to make the total quantity 0.5 c.c.
“ 2	0.3	0.1	0.02 c.c.	
“ 3	0.3	0.1	....	
“ 4	0.3	0.1	0.02	

After incubation for 45 minutes at 37° the bacteria were removed by long centrifugation, washed, resuspended in 0.3 NaCl solution, and in each case there were added suspension of leucocytes 0.1 cc. and NaCl 0.1 c.c. **except that Tube 3 received normal serum 0.02 in NaCl 0.08 in place of NaCl solution only.** After incubation for 30 minutes smears were made and the average number of bacilli taken up in each case determined in the usual way and with the following result:

Mixture 1	4.
Mixture 2	3.8
Mixture 3	**11.**
Mixture 4	0.0

the third experiment, by Dr. D. J. Davis, gives the result of a similar analysis of the promotion by normal human serum of the opsonic effect of immune human serum on a colon bacillus.

The results indicate clearly that opsonic complement is not fixed firmly to the bacterium or corpuscle. The thermostable opsonic element, however, is fixed more firmly as activation is obtainable after washing. Hence the removal of opsonin that Sellards[1] and Centanni[2] obtained by washing treated bacteria may have been more apparent than real, the loss in the amount of phagocytosis being accountable by the removal of the activating substance which appears to remain quite free in the fluid of the phagocytic mixtures. If that be the case this fluid should retain activating properties after the corpuscles or

[1] *Jour. Infect Dis.*, 1908, 5, p. 308.

[2] *Ztschr. f. physiol. Chemie*, 1908, 55, p. 140.

bacteria treated with opsonic serum are removed. Such is the case at least so far as mixtures of dog serum with rat corpuscles are concerned. Indeed, in mixtures of dog serum and corpuscles, dog serum and leucocytes, as well as of serum, corpuscles, and leucocytes, incubated at 37° for 45–60 min., the supernatant fluid upon proper dilution is strongly activating, sometimes fully equivalent in its activating power to that of corresponding amounts of fresh unused serum.

TABLE 9.

THE ACTIVATING POWER OF THE FLUID IN INCUBATED MIXTURES OF SERUM, CORPUSCLES, AND LEUCOCYTES

Mixtures. Each contained 0.2 c.c. 5% susp. rat corp., 0.02 c.c. susp. dog leucocytes, sera as shown below; the total quantity always made 0.6 c.c. by NaCl solution	Percentage of Phagocytic Mononuclears
Heated Serum 0.05	14
Heated Serum 0.05 + Fluid 1 } Amount	45
Heated Serum 0.05 + Fluid 2 } of serum in	50
Heated Serum 0.05 + Fluid 3 } each fluid = 0.005 c.c.	49
Heated Serum 0.05 + Normal Serum 0.005 c.c.	52
Fluid 1 } Amount of	0
Fluid 2 } serum in	0
Fluid 3 } each fluid = 0.005 c.c.	0
Normal Serum 0.005 c.c.	0

NOTE.—Fluid 1 is requisite amount of fluid in a mixture of 0.2 c.c. 5 per cent susp. of corpuscles + normal serum 0.05 c. c. + NaCl sol. 0.15 c.c., incubated at 37° C. for 1 hr. Fluid 2 is requisite amount of fluid in a mixture treated in the same way but with susp. dog. leuc. in place of corpuscles. Fluid 3 is requisite amount of fluid in a mixture of 0.1 c.c. 10 per cent susp. of dog corpuscles + leucocytic susp. 0.1 c.c. + normal serum 0.05 + NaCl sol. 0.15, incubated at 37° C. for 1 hr.

On heating the supernatant fluid the activating property is lost. In this particular case then there is reason to conclude that the complementing or activating element is not fixed either by the corpuscle, by the phagocytes, or by the phagocytes when engaged in taking up the corpuscles. Further experiments designed to reveal something further of the mechanism of activation have not given decisive results and no indication of any direct stimulating effect upon the phagocytes to the extent that it will survive washing in salt solution has been obtained.

THE EFFECT OF HEAT UPON MIXTURES OF BACTERIA AND OPSONIC SERUM.

If the activating element remains quite free in the fluid of the phagocytic mixture, one would expect that heating the mixture to 60° C. for 15–30 min. before adding the leucocytes will destroy this element so that the amount of phagocytosis obtained on adding leucocytes after heating practically would be the same as that obtained on using heated serum from the start.

In their original experiments on the opsonic action of serum, Wright and Douglas[1] found that if the mixture of staphylococci and serum was heated immediately after it was made only a minimal phagocytosis would result. But if the mixture was first digested for 15 minutes at 37° C. and then heated for 15 min. or longer at 60° C. pronounced phagocytosis would result. Bullock and Atkin,[2] Sellards,[3] and others have obtained confirmatory results, the phagocytosis on heating after digestion at 37° C. being in some cases at least somewhat less than in the unheated control mixture. Bullock and Atkin found that after the opsonin in normal serum had united with staphylococci, the mixture of serum and cocci could be heated to 60° C. for several hours without abolition of the opsonic effect. Using serum in dilution of 1:10 Sellards, however, noted a marked reduction in phagocytosis when the mixture of serum and staphylococci was heated after digestion, though not quite so marked as when heated before the digestion. All these experiments were made with human serum and staphylococci. In some of them, notably Bullock and Atkins, very dense suspensions of staphylococci were used. The possibility also suggests itself that the serum in some trials may have contained newly formed thermostable opsonic elements as the product of unrecognized staphylococcus infection. For these and other reasons further observations are desirable and the results of some new experiments are given in Table 10.

TABLE 10.

THE EFFECTS ON THE OPSONIC ACTION OF HEATING MIXTURES OF SERUM AND BACTERIA.

TREATMENT OF SERUM+BACTERIA BEFORE ADDING LEUCOCYTES	PHAGOCYTOSIS			
	Staphylococcus	Streptococcus	Pneumococcus	Anthrax Bacillus
At 58° for 30 min., then at 37° 30 min.	4.	0.2	1.2	35
At 37° for 30 min., then at 58° 30 min.	6.3	0.5	1.8	37
At 37° for 60 min.	12.2	5.	4.	75

NOTE.—The figures represent the number (average) of bacteria per leucocyte in mixtures of equal parts of human serum, bacterial suspension, and leucocytes, except those under Anthrax Bacillus, which represent percentage of phagocytic polynuclears in mixtures containing dog serum and dog leucocytes.

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[1] *Proc. Roy. Soc.*, 1903, 72, p. 357.

[2] *Ibid.*, 1905, 74, p. 380.

[3] *Jour. Infect. Dis.*, 1908, 5, p. 308.

When mixed with dog serum rat corpuscles commonly undergo more or less lysis if placed at 58–60° C. for 15–60 min. Consequently experiments like those just given were made at low temperatures. At 45° no difference could be made out between the more highly heated mixtures and that left at 37°; at 54°, however, the following results were obtained:

1. Serum+corpuscles at 54° 30 min. and then at 37° 30 min	22
2. Serum+corpuscles at 37° 30 min. and then at 54° 30 min	25
3. Serum+corpuscles at 37° 60 min	65

In all these trials the phagocytosis is greatly diminished in the heated mixtures. While the diminution is not quite as marked in the mixtures heated after incubation as in those heated before, yet the results harmonize well with the theory that the activating element remains free in the phagocytic mixture. If this explanation of the facts noted proves to be correct it will establish a fundamental distinction between the mechanism of opsonification and of lysis, because in the latter process it is held that the complement is bound by the amboceptor. Meyer[1] also suggests the possibility of this distinction.

THE FIXATION OF THE THERMOSTABLE OPSONIC ELEMENT BY THE OBJECT OF ITS ACTION.

Because of its bearing upon the opsonic theory itself of the question of fixation of opsonin by the object of phagocytosis I have made a number of additional experiments especially with rat corpuscles and dog serum.

In a phagocytic mixture containing 0.2 c.c. of a 5 per cent suspension of carefully washed rat corpuscles, 0.05 c.c. fresh normal dog serum +0.15 c.c. NaCl solution, and 0.2 c.c. of suspension of dog leucocytes (washed), usually about 40–50 per cent of the mononuclear leucocytes are found to be engaged in phagocytosis after an incubation of 45–60 min. Merely pouring off the supernatant fluid after incubating the corpuscles with serum + salt solution before adding the leucocytes reduces the amount of phagocytic mononuclear cells to 10 or 12 per cent but this reduction is not always materially increased even if the corpuscles after treatment with serum are washed five to eight times or more in many hundred times their volume of salt solution.

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[1] *Berl. klin. Wchnschr.*, 1908, 45, p. 951.

With dog serum heated to 60° C. for 30 min. the initial phagocytosis before washing is about 10–15 per cent. On the addition of 0.005 c.c. of fresh dog serum to corpuscles treated with heated serum at the same time as leucocytes are added the usual amount of phagocytosis given by unheated serum (0.05 c.c.) is often obtained no matter whether the treated corpuscles are washed or not before adding the serum. Approximately the same result is obtained on adding 0.005 c.c. of normal serum to corpuscles treated with normal serum and then washed, indicating that in either case the corpuscles became sensitized to about the same degree by taking up the heat-resistant opsonic element. Sometimes only little phagocytosis is obtained with corpuscles treated with serum and then washed several times in salt solution, but even in such cases the addition of 0.005 c.c. normal serum has given pronounced activation as shown in the results of the experiment that I cite:

Twenty tubes were filled each with 0.2 c.c. of 5 per cent suspension of rat corpuscles, 0.05 of dog serum heated to 60° C. for 30 min., and 0.15 c.c. of NaCl solution, incubated at 37° C. for one hour, and divided into two lots of 10 tubes each. The corpuscles in the first tube of each lot were not washed, but the corpuscles in the second tube of each lot were washed once in about 6 c.c. of salt solution; those in the third tube twice, and so on until the 10th tube, the corpuscles in which were washed nine times. The experiment was completed by adding to all the tubes of lot 1, 0.1 c.c. of suspension of dog leucocytes and 0.1 c.c. of NaCl solution, and to all the tubes of lot 2, 0.1 c.c. of leucocytic suspension and 0.005 c.c. of normal serum in 0.1 c.c. of salt solution, incubating for one hour when smears were made, and the percentage of phagocytic mononuclears determined with the following result:

	LOT 1.	LOT 2.
No. of Washings	No Normal Serum	0.005 c.c. Normal Serum
0	10	40
1	10	50
2	5	52
3	0	32
4	0	46
5	0	40
6	2	50
7	0	32
8	0	48
9	2	50

Controls

NaCl Solution only.............................. 0
0.005 c.c. Normal Serum in NaCl Solution........ 0

When rat corpuscles are treated with normal and heated dog serum as in the foregoing experiments, there occurs distinct agglutination which often is very marked. The agglutination persists practically undiminished even after the agglutinated corpuscles are washed 10 successive times in relatively large quantities of salt solution, a new quantity of solution being used for each washing. We have then in this particular instance good examples of firm union of the heat-resistant opsonic element and of the agglutinin with the body upon which they act.

In harmony with the doctrine of tropisms according to which motile organisms orient themselves in response to chemical and other forces, Centanni[1] assumes that the bacterium after being brought into contact with opsonin eliminates a positively chemotropic substance composed of the opsonin and the opsonophile bacterial receptors. According to the theory this chemotropin is the direct agent of phagocytosis. Centanni holds that in suitable mixtures of pneumococci and opsonic serum this soluble chemotropin may form in a few minutes because the phagocytic index obtained with the bacteria of such mixtures is much lower after the bacteria are washed than before. When washed bacteria again come in contact with opsonic serum new chemotropin is given off as shown by the high phagocytic index. Centanni also finds support for this theory in the fact that opsonic serum after being in contact with pneumococci acquire greater chemotactic power than the control serum as tested by means of Pfeiffer's capillary tubes inserted into the subcutaneous tissue, the chemotactic force of the different mixtures being measured by the height and density of the columns of immigrated leucocytes. In connection with this it may be pointed out that the increased chemotactic force may have been owing to other substances than the hypothetical chemotropin and that no matter what the constitution of the chemotropic substance, it may not have been the actual agent of phagocytosis as claimed by Centanni. His cited phagocytic results are in complete harmony with the nature, combining properties and co-operation of the two elements in opsonic serum as set forth in the preceding pages; the formation of the hypothetical chemotropin, however, is directly contradicted by the results of numerous absorption experiments which show that opsonin, at least the specific heat-resistant element, is removed when serum is treated with suitable bacteria or corpuscles.[2]

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[1] *Ztschr. f. physiol. Chemie*, 1908, 55, p. 140.

[1] Hektoen, *Jour. Infect. Dis.*, 1908, 5, p. 249; Hata, *Ztschr. f. Hyg.*, 1908, 61, p. 81.

CONCLUSIONS.

In most cases serum, normal as well as immune, owes its full opsonic effect to a specific thermostable element which unites firmly with the object of its action and to a thermolabile element which seems to remain free in the fluid.

Neufeld's claim that the opsonic action of normal serum in general is caused by the lytic amboceptor and complement and that of immune serum largely by entirely different substances (bacteriotropin and hemotropin) is not established beyond question. On the other hand the view that the opsonic substances in most normal and immune sera are specific and identical, differing only in quantity, harmonizes well with the established facts in regard to these substances as well as in regard to antibodies in general. Hamilton's simple thermostable opsonic substance for certain pseudodiphtheric bacilli answers the requirements of Neufeld's hypothetical bacteriotropins but it occurs, however, in normal as well as immune sera.

In so far as a specific thermostable element is an essential agent of opsonification and phagocytosis the opsonic theory is upheld because it is shown that opsonification is the function of an independent unit in the serum and not of the serum as a whole.